Disorders of Erythropoiesis

Quantitative assessment of the transcriptional impact of mutations in the 3'untranslated region of the human β-globin gene: application to the +1480 C→G mutation

The +1480 C→G mutation in the 3' untranslated region (UTR) of the human β-globin gene has been associated with β-thalassemia. A previously validated *in vitro* expression model used to assess the transcriptional impact of this 3'UTR mutation demonstrated that it has no effect on β-globin gene expression.

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β–thalassemia is a genetic disorder characterized by the reduced production of β-globin chains, due to alterations within coding and non-coding sequences of the β-globin gene.[1] Over the past decades, a wide spectrum of mutations associated with β-thalassemia have been considered mainly in the light of clinical and biological data from affected patients and families.[2,3] Whether non-coding mutations are truly involved in the reduced production of β-globin chains or are simple polymorphisms does, however, remain a controversial issue.[4] While several *in vitro* models have been developed to assess the transcriptional impact of these mutations, most of them are only able to assess the relationship between promoter mutations and thalassemia phenotype.[5] Recently, we designed an *in vitro* assay to assess the transcriptional impact of mutations in any part of the human β-globin gene, including in the 5' and 3' untranslated regions (UTR).[6]

Of note, the competitive reverse transcriptase polymerase chain reaction (RT-PCR), initially validated by our group to accurately quantify the transcriptional impact of such genetic alterations, led to an easier and less time-consuming quantitative Taqman real-time RT-PCR.[7] The same methods were then used to clarify the transcriptional impact of the +1480 C→G mutation, which occurs at nucleotide 6 after the termination codon (*term*+6) within the human 3'UTR β-globin gene. This 3'UTR mutation has been associated with silent β-thalassemia in Greek families.[8] Construction of the β-globin expression vector, production of the recombinant pBLG plasmids of interest by directed mutagenesis and fragment exchange, stable transfection of the recombinant pBLG in MEL cells, and subsequent quantification of human β-globin mRNA expression have been previously described.[6]

Wild-type β-globin and the -30T→A variant were tested in parallel as controls. For each construct, transfection of MEL cells was repeated three times on different days. Expression results of all constructs were tested concomitantly with both methods on the same cDNA. All statistical analyses were performed with the SPSS statistical package, release 12.0 for Windows (SPSS, Inc.) as previously described.[6] β-globin expression values for various constructs were compared using Student's t-test. The threshold for statistical significance was set at 0.05.

As shown in Figure 1, the expression data of wild-type and +1480 C→G constructs were comparable (p>0.05), irrespective of the quantitative method used. In contrast,

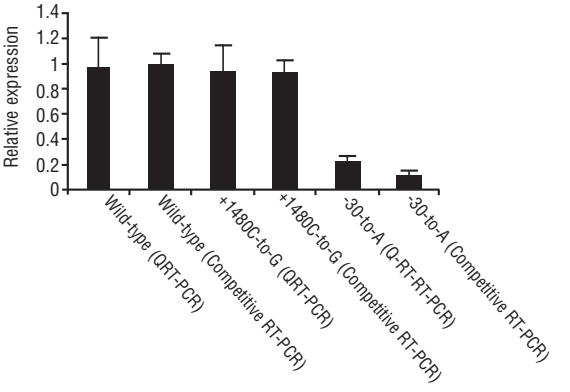

Figure 1. Relative (mean ± standard deviation) expression of human β-globin mRNA in the MEL cell line stably transfected with constructs carrying either wild-type, +1480 C→G, or -30 T→A mutated human β-globin: quantification by real-time quantitative RT-PCR (QRT-RT-PCR) and competitive RT-PCR.

the -30T→A control plasmid expressed very low levels of human β-globin, ranging between 30 and 10%, as already recorded previously with both methods.[6,7] However, discrepant data were previously obtained by others with the +1480 C→G variant.[9] Using, like us, MEL cells stably transfected with a recombinant expression vector containing the μLCR and full length human β-globin with the +1480 C→G mutation, the authors showed mRNA expression decreased to 52 to 60% that of the wild-type control. Unfortunately, no known thalassemic mutation was used as a control to strengthen the validity of the results. Accordingly, discrepancies between their results and ours could be explained by methodological differences. Many factors may affect the reproducibility of quantitative assays using transfected MEL cells (number of cells collected for quantification, reproducibility of the erythroid differentiation, reproducibility of total RNA extraction, etc.).[6] In the current study, these factors were carefully controlled. Moreover, two distinct carefully controlled quantitative methods performed with the same cDNA generated similar results. Finally, highly reproducible data were obtained with the –30T→A control plasmid, and these data were also identical to those obtained in our previous trials with the same methods.[6,7]

Despite the lack of transcriptional impact as demonstrated here, the +1480 C→G mutation is clearly associated with silent β-thalassemia. To reconcile the contribution of this 3'UTR mutation in the pathogenesis of thalassemia and the lack of transcriptional impact, we point out that the 3'UTR of eukaryotic genes were recently shown to contain specific sequence elements that can modulate translational efficiency.[10] Accordingly, it could be inferred that the +1480 C→G mutation impairs translation efficiency, although this point remains to be clarified.

In conclusion, our study shows that the quantitative Taqman real-time RT-PCR method can be successfully used to assess the transcriptional impact of mutations in noncoding regions of the β-globin gene in general, and in the 3'UTR region in particular. Our study confirms unambiguously that the 3'UTR +1480 C→G mutation has no significant impact on the transcription of the human β-globin gene. The potential role of translational

regulators in the 3'UTR region of eukaryotic genes and our current data therefore support the hypothesis that the +1480 C→G mutation affects the translation of corresponding mRNA rather than decreasing its steady-state stability.

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